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Layer-by-layer assembled nanocoatings of human platelet's lysate and marine-origin polysaccharides trigger pro-angiogenic behaviour

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Introduction: Several growth factors (GFs) participate in the regulation of cell proliferation, migration, differentiation and apoptosis. GFs such as VEGF-A and FGF-b are essential to trigger the angiogenic cascade that is crucial for the subsequent steps of new tissue formation.[1] Human Platelet's lysate (hPL) has been used as an autologous source of GFs.[2] Herein, we investigated whether marine-origin polysaccharides are able to attract and stabilize pro-angiogenic GFs from PL and activate endothelial cells by providing pro-angiogenic cues.
Materials and methods: κ-, ι-, λ-carrageenan (Car), alginate, chitosan and heparin were purchased from Sigma-Aldrich. Human PL was obtained as described elsewhere[3]. The interaction of mentioned the polyelectrolytes (PEs) with PL was assessed by QCM-D (Q-sense). The thickness of the nanocoatings was measured by ellipsometry and VEGF-A and FGF-b binding quantified by ELISA (Peportech). Human umbilical vein endothelial cells (HUVECs) were cultured in M199 culture media supplemented with 20%FBS and ECGS. Cells were seeded on 48-well-plates previously modified with the nanocoatings prepared by Layer-by-Layer assembling, i.e. by the alternating deposition of each of the mentioned PE with diluted PL (1, 3 and 6 bilayers), in the absence of ECGS and with 10% serum. The effect of the VEGF-A and bFGF on HUVECs was assessed in cultures established with 150 or 200 nM of FGF/VEGF Receptor Tyrosine Kinase Inhibitor (Santa Cruz Biotechnology).
Results: The thickness of nanocoatings varied between 30 and 45 and the GFs binding was quantified. The more sulfated PE's, heparin/PL and λ-Car/PL, were able to induce the formation of tube-like structures after 20 hours of culture (Figure 1). An increase of tube-length was observed with increasing number of bilayers. When the binding of the angiogenic GFs to the VEGF/FGF receptors is inhibited, the formation of tube-like structures is reduced or does not occur, depending on the concentration of the inhibitor.

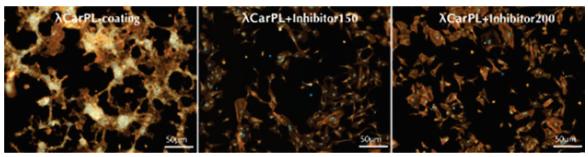

Figure 1. HUVECs cell morphology after 20 hours (blue-nucleus, orange-cytoskeleton) on λ-Car/PL coatings in absence (left) or presence of 150 nM (middle) and 200 nM (right) of inhibitor.

The cells were able to proliferate on the nanocoatings that have induced tube-like structures formation, especially on ι-carrageenan/PL. However with proliferation slowed down in absence of ECGS, tube-like structures can be observed after 4 days in culture.
Discussion and conclusions: Nanocoatings composed by sulfated marine-origin polysaccharides and hPL bio-activate endothelial cells inducing the formation of tube-like structures. The formation of the tube-like structures, which depended on the PE and number of bilayers, was achieved after 20 hours of incubation and was mediated by the VEGF/FGF. The combination of hPL with these PEs may be an efficient and simple method to introduce pro-angiogenic cues in any 2D/3D cell-material interface and improve tissue regeneration.
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PP34
Bioactive inorganic microcarrier with protein delivery designed for bone tissue engineering

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Introduction: Development of bioactive microcarriers that allow 3D culture of stem cells is important for bone tissue engineering. Aim is to engineer microcarriers with bioactive composition and growth factor-delivering capacity which is ultimately effective for osteogenesis of stem cells and bone regeneration. We propose a novel bioactive microcarrier composed of silica-based bioactive glass which is produced by a sol-gel process.
Materials and methods: The microcarriers were prepared by water in oil emulsion, taking advantage of the sol-gel reaction of tetraethyl orthosilicate. Microcarriers were doped with different calcium ions from 0 to 30. Specific surface area, pore size and zeta potential were measured. Model protein was encapsulated in two different compositions to observe calcium effect on release profile. In vitro bioactivity was performed immersing samples in SBF. The biocompatibility was determined in vitro and in vivo. Basic Fibroblast Growth Factor (bFGF) was encapsulated within the microcarriers and the effects on cell proliferation were analyzed.
Results: Highly mesoporous structure was created with mesopore sizes of 2.5~6.3 nm and specific surface area of 420~710 m^2/g, which was highly dependent on the Ca concentration. Cyt C profiled an almost zero-order kinetics presenting slight increase in release rate, presumably due to the larger pore size. The presence of Ca accelerated the formation of apatite on the surface of the microcarriers. Cells cultured on the bioactive microcarriers adhered and spread well and proliferated actively. In vivo study showed the tissue compatibility of the microcarriers. Near zero order kinetics with releasing period of over a month was shown for bFGF release. Cells adhered and proliferated to significantly higher degree on the bFGF-loading samples, demonstrating an effective role of bFGF released from the microcarriers.
Discussion and conclusions: Bioactive silica-based sol-gel derived microcarriers demonstrated high mesoporosity with tunable pore size and volume and surface area. They also had self-hardening ability to safely incorporate biological molecules with long-term delivery over months, and showed excellent biocompatibility to enable cell growth and tissue reactions. Based on these, the microcarriers may be potentially useful for bone tissue engineering.
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